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Isolation of β-carotene from Palm (*Elaeis guineensis* Jacq.) Oil Using Transesterification-adsorption-desorption Method and its Characterization

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Abstract: A combination of adsorption and desorption method of a transesterification product in the process of isolation of β-carotene has been successfully carried out. Palm oil was transesterified using sodium hydroxide and methanol. The product was then adsorbed using kaolin and celite as comparison but kaolin was selected as a better adsorbent. The product was then desorbed using Soxhlet extractor with ether or maceration with n-hexane. Desorption results was purified by column chromatography and its purity was tested by HPLC. β-carotene obtained was further characterized by UV-Vis spectrophotometry, IR spectroscopy and NMR spectroscopy. The weight of filtrate and concentration of carotenoid in the sample obtained using Soxhlet's method were 39.37 g and 184.99 ppm, whereas maceration method yielded 39.54 g and 160.66 ppm, respectively. The weight of purified β-carotene from soxhletation and maceration obtained by column chromatography were 17.43 and 16.80 mg, respectively. Isolation of β-carotene using maceration as desorption method produced β-carotene weight of 86.22 mg with 98.31% purity as tested with HPLC, whereas the isolation with soxhletation as desorption method resulted in 111.11 mg β-carotene with 97.12% purity. The results of characterization using UV-Vis spectrophotometry showed β-carotene's absorption maxima at 450 and 477 nm. Following characterization using IR spectroscopy, absorption frequencies were shown at 2923.56 and 2854.13 cm^{-1} (aliphatic C-H), 1650.77 cm^{-1} (aliphatic C = C). 1H NMR spectrum showed a multiplicity doublet on chemical shifts at 6.26 and 6.63 ppm as the specific peak of β-carotene. In conclusion, β-carotene from palm (*Elaeis guineensis* Jacq.) oil using transesterification-adsorption-desorption method has been succesfully isolated and characterized.

Key words: Palm oil, transesterification, adsorption, desorption, kaolin, β-caroten

INTRODUCTION

Palm (*Elaeis guineensis* Jacq.) is one group of plants derived from African palm. The economic value of the tree lies with the production of palm oil in the fruit mesocarp. Derivative products from palm oil have many prospects for beneficial developments. Until recently, palm oil in Indonesia has not been processed into other derivatives that have high economic value. One of the palm oil derivative products that can be used is carotenoids. Carotenoids content of palm oil ranges from 630 to 700 ppm (Zeb and Mehmood, 2004). Palm oil contains 10 to 200 times more carotenoids than carrots, green leafy vegetables and tomatoes (Priyadarshani and Chandrika, 2007).

Carotene pigments is constituted by α, β, γ-carotene and lycopene. The compound acts as an important substance that is needed by the body to further processing into vitamin. Carotenoids, whether provitamins A or not, have been credited with other beneficial effects on human health: Enhancement of the immune response and reduction of the risk of degenerative diseases such as cancer (Schwarz *et al*., 2008), cardiovascular disease (Riccioni, 2009; Palozza *et al*., 2008), cataract (Vu *et al*., 2006) and macular degeneration (Moeller *et al*., 2006). The human body has the ability to convert large amounts of β-carotene into vitamin A (retinol).

Provitamin A activity is very high in β-carotene whereas α and γ-carotene have about 50-54% and 42-50% as much, respectively. Approximately 25% of β-carotene absorbed from the intestinal mucosa remains in the intact form, while 75% is converted to retinol (vitamin A) with the help of the enzyme 15,

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15'-β-carotenoid oxygenase (Zeb and Mehmood, 2004; Priyadarshani and Chandrika, 2007). Judging from this data, therefore, development of β-carotene isolation is urgently needed.

Over several decades, various methods have been developed in order to recover carotenoids in Crude Palm Oil (CPO). These include saponification (Nesaretnam *et al*., 2007), selective solvent extraction (Chiu *et al*., 2009) transesterification followed by distillation (Buckl *et al*., 1999) and adsorption (Ooi *et al*., 1994). Adsorption without prior chemical reaction has been reported by Othman *et al*. (2010). However, the separation process showed more difficulties in purification in comparison to prior transesterification. A combination of transesterfication, adsorption and desorption may lead to a better result. The selection of adsorbent was based on the ability to adsorp carotenoid components and release it during the desorption process.

In this study, two types of adsorbent were used, namely kaolin and celite. Kaolin is used in this study due to its abundant availability. Therefore, production in a large-scale will not face a problem of raw materials adsorbent. This study was aimed to isolate β-carotene in palm oil through transesterification process and through a combination of adsorption and desorption methods using Soxhletation and maceration. Adsorption method was optimized to choose between kaolin and celite as comparison. Isolated β-carotene wasthen analyzed by high-performance liquid chromatography to check for its purity. Furthermore, β-carotene was identified and characterized using physicochemical methods such as UV-Vis spectrophotometry, infrared spectroscopy, nuclear magnetic resonance spectroscopy. In addition, the levels of β-carotene were determined using UV-Vis spectrophotometer.

MATERIALS AND METHODS

Materials: Palm (*Elaeis guineensis* Jacq.) oil, standard of β-carotene (Sigma-Aldrich; 1,600,000I U/g), sodium hydroxide (Merck), methanol pro analysis (Merck), kaolin, sulfuric acid (Merck), distilled water, n-hexane (Brataco chem.), chloroform (Brataco Chem.), silica gel H (Merck), Whatman paper No. 3, acetone (Merck), potassium bromide (Merck).

Apparatus: Ultraviolet-Visible (UV-Vis) Spectrophotometer (Beckman DU650i), Fourier Transform-Infrared (FT-IR) Spectrophotometer (Jasco FT-IR 4200), High Performance Liquid Chromatography (HPLC) (Hewlett-Packard series 1100) and Nuclear Magnetic Resonance (NMR) Spectrometer (Agilent 500 MHz).

Procedure

Transesterification reaction: Transesterification procedure is a modification of that previously done by Buckl *et al*. (1999) A total of 100 g of palm oil was weighed and transferred into a 250 mL round flask. An amount of 0.592 g of NaOH was dissolved in 54.2 g of methanol pro analysis and was added into the round flask. The round flask was then placed in a reflux apparatus. The mixture was stirred using a stirrer at a temperature of 60°C. After 1 h, the stirrer was turned off and the mixture was transferred into a separating funnel and allowed to separate into two layers, Fatty Acids Methyl Ester (FAME) and glycerol layers. The FAME layer was then separated. Sulfuric acid of 0.33 M was added until a neutral pH was achieved. The FAME layer was then washed twice with distilled water.

Optimization of the adsorption process: Ten grams of adsorbent was added to 30 mL of FAME in an Erlenmeyer flask. The adsorbents used were celite and kaolin. Adsorption process was carried out at three temperature conditions, namely 40, 50 and 60°C, each tested within three different time durations: 20, 40 and 60 min. Stirring speed for each condition was the same.

Adsorption and screening for adsorbents: Twenty five grams of adsorbent was added to 75 g of FAME, with kaolin as adsorbent. Adsorption process was carried out at a temperature of 60°C for 1 h. Stirring speed for each condition was identical. Samples were filtered using Whatman paper no. 3 and a vacuum pump to obtain a dry adsorbent residue. Filtration and adsorption were repeated until a clear and colorless filtrate was obtained.

Desorption of adsorbent: Desorption process of adsorbent were carried out using soxhletation and maceration. For Soxhlet method, ether was used in desorption at 40°C whereas for maceration, n-hexane was used. The desorption was done to obtain a clear orange and colorless solvent from soxhletation. With maceration, the desorption was done by soaking the adsorbent repeatedly in a solvent for 24 h until a clear and colorless product was obtained.

Purification of desorption results: Initial purification process of β-carotene was carried out using a classical column chromatography. The stationary phase used was silica gel 60 with n-hexane:chloroform (6:4) as the mobile phase. Further purification was done by the separation of fatty acid β-carotene which was done by adding acetone

to the sample and stirring for 30 min. The sample was then kept at -20°C for 5 h (Khachik, 2006). Samples were filtered using 0.45 μm membrane in cold condition. The filtrate was evaporated using nitrogen gas.

Characterization of β-carotene: Characterization was done by UV-Vis Spectrophotometer, FT-IR Spectrophotometer and NMR Spectrometer, preceded by purity confirmation using HPLC. Purity test was conducted by ODS column 3 GI SCIENCE ® RP-18 (150 ×4.6 mm, 5l m). Elution system was a gradient elution, in which in the 0-7 min period 100% methanol system was used followed by methanol: acetonitrile: dichloromethane (40:55:5) in the 7-42 min period. The mobile phase flow rate was 1.2 mL min^{-1}. The injection volume was 20 mL. The experiments were conducted at a temperature of 25.7°C. Absorption was measured at a wavelength of 450 nm. Elution time for identification was done for 45 min. In UV-Vis spectrophotometry and FT-IR spectrophotometry, the β-carotene sample was compared to the standard. The isolate of β-carotene was then further characterized using 1H-NMR.

RESULTS

Adsorption optimization process was performed at three temperature conditions, namely 40, 50 and 60°C with adsorption times of 20, 40 and 60 min at each temperature, respectively. The adsorption effectiveness was compared when kaolin or celite was used as adsorbent (Fig. 1).

The decrease in carotenoid concentration following soxhletation and maceration is presented in Fig. 2.

Identification was done by using UV-Vis spectrophotometer by overlaying the sample with the standard β-carotene. The sample and standard were dissolved in n-hexane and the overlaid spectra were obtained as given in Fig. 3.

Figure 4 depicts FT-IR spectra of the samples obtained from soxhletation and maceration together with the standard β-carotene.

Purity test was done using HPLC by measuring the chromatogram at the wavelength of 450 nm as done by Lyan *et al.* (2001) and Inbaraj *et al.* (2006) at the identical wavelength. Retention time for standard β-carotene was recorded to be 33 min. Figure 5 shows the purity of the sample with maceration process.

Results of 1H-NMR spectra prediction using software ChemDraw 13.0 showed some peaks from the chemical structure of β-carotene, as presented in Table 1.

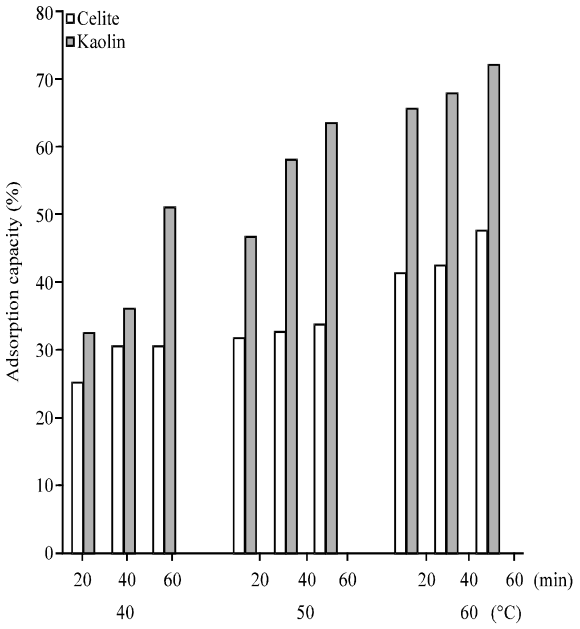

Fig. 1: Comparison of adsorbability between celite and kaolin

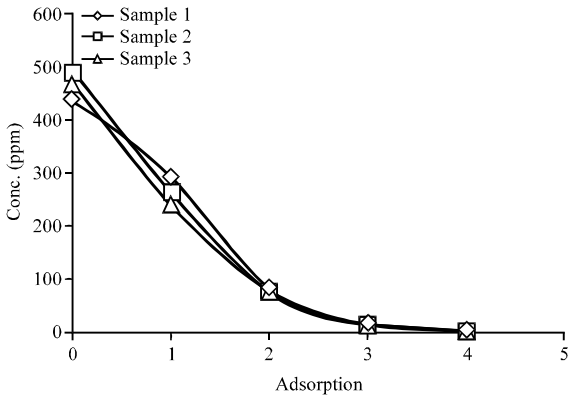

Fig. 2: Decrease in carotenoid concentration with repeated adsorption

Table 1: Comparison between predicted and sample chemical shift sample purified by maceration

Predicted chemical shift (ppm)	Multiplicity	Sample chemical shift (ppm)	Multiplicity
1.25	Singlet	1.27	Singlet
1.57	Triplet	-	-
1.74	Multiplet	-	-
1.82	Singlet	1.86	Singlet
1.96	Triplet	2.02	Triplet
2.21	Singlet	2.31	Singlet
6.23	Doublet	6.26	Doublet
6.51	Doublet	6.63	Doublet
	DoubletDoublet	6.64-6.65	DoubletDoublet

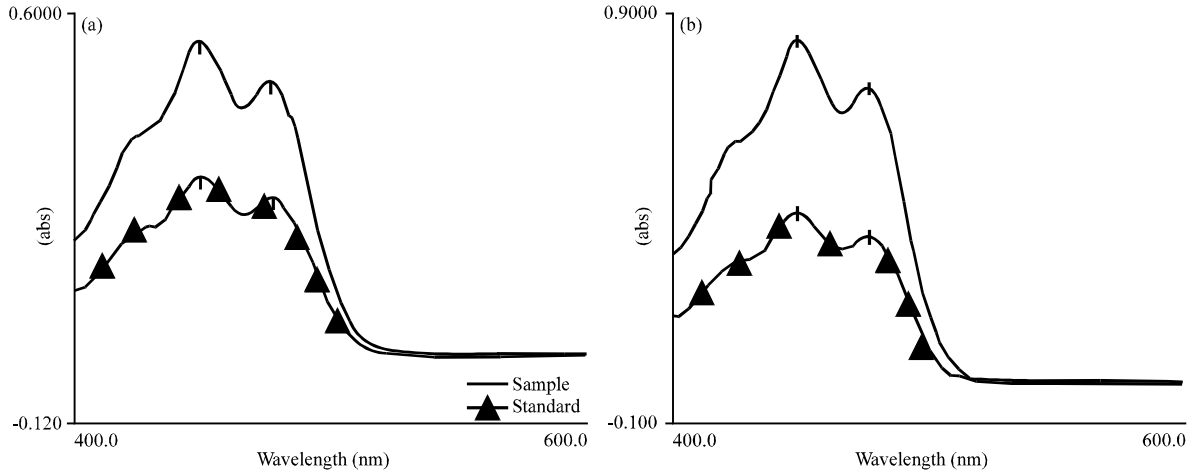

Fig. 3(a-b): Overlaid UV-Vis spectra (a) Sample resulted from purification with soxhletation and (b) Sample purified with maceration

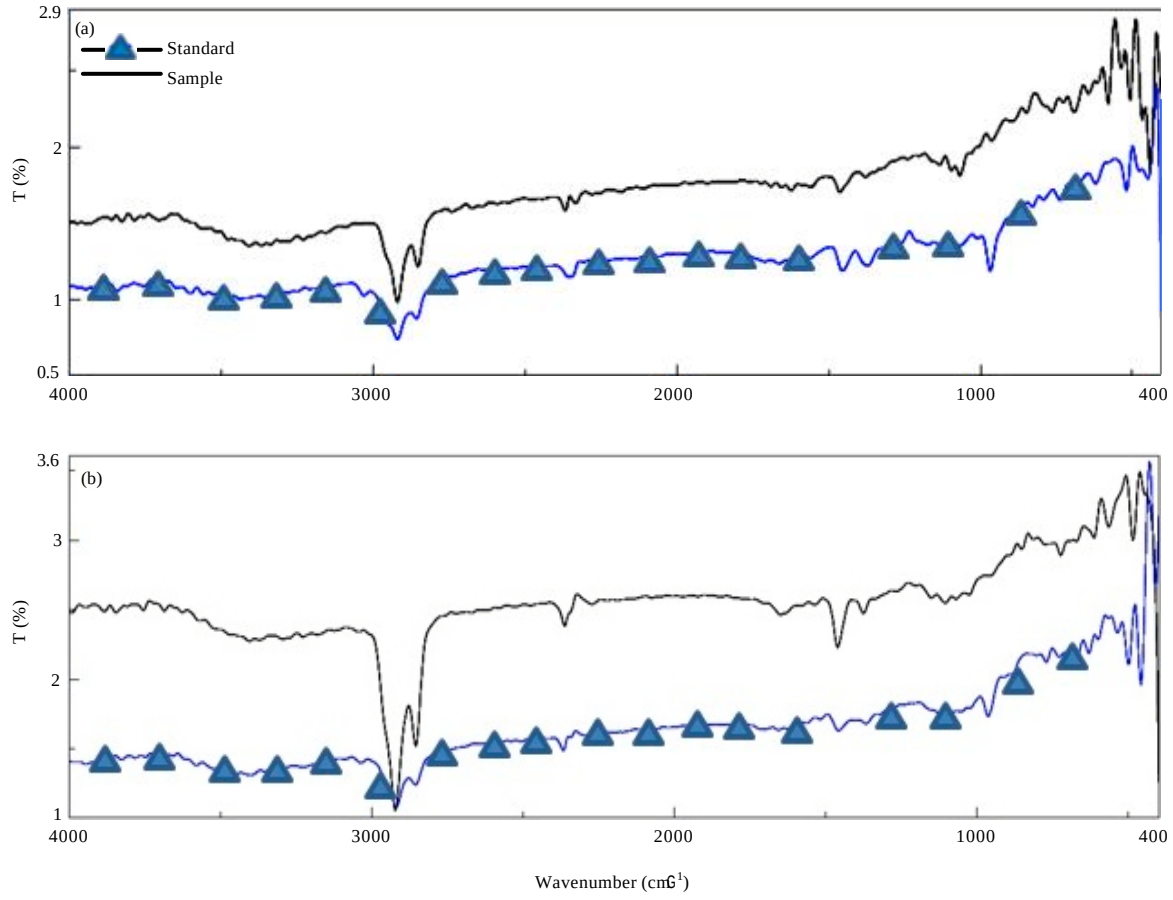

Fig. 4(a-b): Overlaid IR spectrum (a) Sample purified with soxhletation and (b) Sample purified with maceration

DISCUSSION

Selection of temperature in a range of 40-60°C was based on the stability of carotenoids which decreased by 5% at 70°C (Fratianni *et al*., 2010). Furthermore, to avoid longer exposure to heat, the upper limit was set to 60 min. with 20 min interval. Adsorption capability is obtained by dividing the concentration of carotenoids in the adsorbent residue by initial concentration of carotenoids contained in FAME. The larger the value, the better adsorption capacity, which means that the components adsorb carotenoids contained in FAME. As shown in Fig. 1, the adsorption capacities of both celite and kaolin increase with temperature and time. The optimum adsorption was obtained using kaolin as an adsorbent at a temperature of 60°C for 1 h, yielding 72.03% adsorption.

Carotenoid adsorption process of transesterification products is the selective adsorption of the reddish yellow pigment on the surface of the adsorbent pores. As presented in Fig. 2, the concentration of carotenoids in the FAME decreases, indicating the increasing number of components of the carotenoids in the FAME adsorbed by the adsorbent. After the fourth adsorption, carotenoid levels remaining in the filtrate was 1-2 ppm decreased from the initial value of 420-450 ppm.

Desorption of the carotenoid-containing adsorbent was performed by soxhletation using ether and maceration in n-hexane. After the desorption, clear extracts obtained were collected and evaporated using nitrogen gas. Nitrogen gas was selected due to the nature of the carotenoid components that are not stable against light, oxygen and heat. Desorption results obtained were 39.37 and 39.54 g after soxhletation and maceration, respectively. Carotenoid concentration in concentrated extract obtained was measured using a UV-Vis spectrophotometer and it was found that carotenoid concentrations measured as β-carotene were 184.99 and 160.66 ppm with soxhletation and maceration, respectively.

Table 2: Interpretation of infrared spectra of samples

Functional group	Wavenumber (cm^{-1})
C-H stretch	2923.56
	2854.13
Aliphatic C = C stretch	1650.77
Methyl C-H bend	1461.78

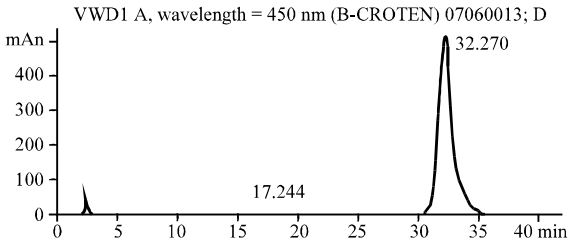

Fig. 5: HPLC chromatogram of sample purified by maceration

For purification, the classical column chromatography system was used. Following evaporation and precipitation, a total of 4.5 g sample extract yielded 17.4 mg product with soxhletation as pre-precipitation process and 16.8 mg when the preceding process was maceration. From a total of 39 g condensed filtrate as desorption result, the column chromatography yielded 154.51 and 149.18 mg products from the soxhleted and macerated samples, respectively. On the other hand, the fatty acid separation produced 86.2 mg product from the soxhleted sample and 111.11 mg from the macerated sample.

It is found that although the spectral profile of the sample is similar with that of the standard and they have the same shoulders at the wavelengths of 450 and 477 nm. These findings suggest that there is β-carotene in the sample. On the other hand, it has been shown that the absorbance increases with increasing the concentration of the standard and the spectral profile of the sample can perfectly overlap with that of the standard by changing the concentration of the standard (Karnjanawipagul *et al*., 2010). Therefore, a perfect overlap of the spectral profiles in Fig. 3 could be achieved by increasing the concentration of the standard.

The FT-IR spectrum of standard β-carotene has three distinctive peaks at 2923.56 and 2854.13 cm^{-1} (aromatic CH stretch and aliphatic CH stretch, respectively), 1650.77 cm^{-1} (aliphatic C = C stretch), which is abundantly present in the structure of β-carotene. Interpretation of the FT-IR spectra of samples from soxhletation and maceration is presented in Table 1. The third peak 1650.77 should be present in a sample and the result showed three peaks, indicating that the samples contained β-carotene. This result is in the area with the study that was done by Zaibunnisa *et al*. (2011) that showed peaks at 3420, 2928, 1718 and 963 cm^{-1} (Table 2).

Results of 1H-NMR spectra prediction using software ChemDraw 13.0 showed some peaks from the chemical structure of β-carotene, as presented in Table 2. Although, the chemical shift of the sample was not the same as that of the predicted, they shared common multiplicity. This might be due to differences in the measurement conditions for the sample and predicted spectrum by the ChemDraw 13.0. In addition, the samples might still contain other peaks that are absent in the predicted, which might be indicative of saturated fatty acid content in the sample.

Structure of β-carotene has a characteristic of aliphatic of conjugated double bond. This bond will appear at about 5-6 ppm. The NMR spectra of samples purified with soxhletation and maceration showed typical β-carotene spectrum characterized by chemical shift around 6.23 and 6.51 ppm, despite with small intensity which might be attributable to the low concentration of β-carotene. The spectrum showed similar peak with the study that has been done by Hruszkewycz (2009) and Cardoso *et al*. (1996). The residue of solvents $CDCl_3$ and $CDCl_3$ appeared at 7.27 ppm chemical shift. At 0.8 to 2.3 ppm chemical shift, the peaks formed were not too clear and had high integration. This might be due to the persistence of fatty acid impurities which helped to improve the intensity of chemical shift around 0.8 to 1.4 ppm.

Isolation of β-carotene by maceration method using desorption process produced 86.22 mg β-carotene with 98.31% purity as assessed by HPLC. Meanwhile, the isolation with soxhletation produced 111.11 mg β-carotene with 97.12% purity. The produced β-carotene gave characteristic peaks at 450 and 477 nm as confirmed by the UV-Vis spectrometry. The FTIR spectroscopy indicated that the produced β-carotene has functional groups with peaks at 2923.56 and 2854.13 cm^{-1} (aliphatic C-H stretch), as well as 1650.77 cm^{-1} (aliphatic C = C). 1H NMR spectrum showed a Doublet multiplicity on chemical shift of 6.26 and 6.63 ppm which are the typical peaks of β-carotene structure.

In conclusion, β-carotene from palm (*Elaeis guineensis* Jacq.) oil using transesterification-adsorption-desorption method has been succesfully isolated and characterized.

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